(c) encoded by a nucleotide sequence capable of hybridizing to (a) or (b) in a hybridization solution comprising 6XSSPE and 0.5% SDS at 65°C overnight followed by washing with a solution comprising 2XSSPE and 0.1% SDS for 10 minutes twice,

wherein said nucleotide has a sequence encoding a polypeptide having amylase enzyme activity.

REMARKS

Claims 25-43, 123-128, 147, and 150-154 are pending. Claims 25-37, 123, 124 and 128 are amended and claims 150-154 are added. Claims 70- 122, 129-146, 148 and 149 are canceled without prejudice or disclaimer for filing in one or more divisional applications. The amended claims are fully supported by the specification and claims as originally filed. New claims 150 has support in Example II-1 and on page 91, l.24-page 95, l.10; Tables 12, 13 & 14; pages 98, 102 & 106. Claim 151 has support in original claim 28. Claim 152 has support for section (b) in the specification on page 54, ll.22-26; page 54, l.31-page 55, l.6 and for section (c) on page 173, ll.28-31. Further, claim 153 has support for section (b) on page 54, ll.26-30; page 55, ll.6-14 and for section (c) on page 173, ll.28-31. The claims have been made to more clarify the present invention and is not intended to limit its scope. The specification has been amended to insert SEQ ID NOS. No new matter has been added to the specification.

Election/ Restriction

The Examiner has maintained the restriction requirement and has refused to examine the claims of Group II directed to a method of making the amylase of Group I with claims 25-37 and 123-128 of elected Group I. Applicant again requests that the Examiner reconsider this request but should the Examiner maintain his position, applicants point out that rejoinder of process of making claims commensurate in scope with product claims found allowable is permitted under the *Ochiai* guidelines, and such rejoinder is requested. This request applies to claims 38-43. In regard to claim 147, it depends from claim 123 and should be examined with the claims of Group I. Reconsideration of the restriction requirement and pending claims is respectfully requested.

Priority

The Examiner's acknowledgement of the applicants' foreign priority and the filing of the certified copy in the parent application, U.S. Serial No. 6,391,595 is noted.

Sequence Listing Compliance

Applicants enclose a copy of a revised Sequence Listing and have amended the specification and claims to insert the SEQ ID NOS. Applicants submit that they have satisfied the Examiner's request.

Rejections under 35 U.S.C. 112, second paragraph

The Examiner alleges that claims 25-37 and 123-128 as allegedly being indefinite in the recitation of specific language. Applicants have amended the claims to clarify the present invention and it is believed that these clarifying amendments address the Examiner's bases for these rejections, and withdrawal of these rejections is kindly requested.

Rejections under 35 U.S.C. 112, first paragraph

Alleged Lack of Enablement

Claims 25-37 and 123-128 are rejected under 35 U.S.C. § 112, first paragraph because the Examiner alleges that while the claims are enabled for an enzyme with SEQ ID NOS: 6 or 8, the specification does not reasonably provide enablement for all variants of such polypeptides or such polypeptides isolated from all other sources.

Applicants respectfully traverse this rejection and submit that the specification describes, for example, in Examples II-2 to II-4 the isolation of the amylase of the present invention from three sources belonging to the genus *Sulfolobus* including purification and activity measurement thereof. Additionally, Tables 12-14 show the enzyme activity, i.e., the trehaloseoligosaccharide-hydrolyzing activity, of the objective amylase obtained in the respective purification steps in each of the examples. Further, the activity measurement is described in Example II-1 along with the definition of the trehaloseoligosaccharide-hydrolyzing activity. Table 13 specifically shows that the objective amylase purified by DEAE ion-exchange chromatography has a specific activity of 10.6 units/mg.

The Examiner seems to take the position that the specification discloses only two species of polypeptides, i.e., the polypeptide comprising an amino acid sequence of SEQ ID NO: 6 and the polypeptide comprising an amino acid sequence of SEQ ID NO: 8. However, the specification does describe, modified polypeptides, for example, the modified polypeptides which are derived from SEQ ID NO: 6 or 8. See the paragraph bridging pages 56 and 57 of the specification.

Under the heading "A Gene Coding for the Novel Amylase" at pages 53-56, the base sequence of SEQ ID NO: 5 encoding SEQ ID NO: 6 (e.g., the base sequence comprising a sequence from 642 to 2315 of SEQ ID NO: 5) and the base sequence of SEQ ID NO: 7 encoding SEQ ID NO:8 (e.g., the base sequence comprising a sequence from 1176 to 2843 of SEQ ID NO: 7) are described as examples of suitable DNA sequences coding for the amylase of the present invention, i.e., the DNA sequence coding for the amino acid sequence of SEQ ID NO: 6 or 8.

In this regard, the specification further describes, for example, in Examples II-15 and II-20, the determination method for the amino acid sequences of the amylase of the present invention; in Example II-16, the preparation method for chromosome DNAs; in Example II-17, the expression cloning method for the gene coding for the amylase of the present invention; in Example II-18, the determination method for the gene coding for the amylase of the present invention; in Example II-19, the production method for the recombinant amylase in a transformant; in Example II-21, the preparation method for DNA probes; in Example II-22, the cloning method for the gene coding for the amylase of the present invention, especially including hybridization method (page 173, lines 28-31); in Example II-23, the homology determination methods for SEQ ID NO: 6 vs. NO: 8 and also for SEQ ID NO: 5 vs. NO: 7; and, in Example II-24, the hybridization tests among the genes coding for the amylase of the present invention.

The specification further describes, for example, at page 13, lines 13-28, and demonstrates, for example, in Example II-6, that the present purified amylase is capable of acting on a trehaloseoligosaccharide which is derived from a maltooligosaccharide by transforming the first linkage from the reducing end into an α-1,α-1 linkage, and hydrolyzing specifically the α-1,4 linkage next to the α-1, α-1 linkage to liberate α,α-trehalose in a high

yield, i.e., capable of hydrolyzing trehaloseoligosaccharide specifically at the α-1,4 linkage between the second and third glucose residues from the reducing end side, as well as hydrolyzing the α-1,4 linkage in the molecular chain of starch or starch hydrolysate, to liberate α,α-trehalose, which hydrolyzation has in no way been attained by any conventional amylases.

Applicants submit the explanations above provide evidence that the pending claims are fully supported by the present specification and it is requested that the Examiner withdraw this rejection

Alleged Lack of Written Description

Claims 25-37 and 123-128 are rejected as allegedly not adequately described in the specification to convey the invention to a person skilled in the art. Applicants respectfully traverse this rejection on similar grounds as argued above. The specification on pages 53-63 describe the genes encoding the amylase of the present invention and refers to equivalent sequences to the identified SEQ ID NOS; 6 and 8. Specifically, in the paragraph bridging pages 56 and 57, the term "equivalent sequence" is described as the amino acid sequence of SEQ ID NOS: 6 or 8 but containing insertions, replacements or deletions of some amino acids yet retains amylase activity.

Applicants submit that the present specification provides sufficient written description to allow one skilled in the art to carry out the present invention. The specification provides the information and amylase sequences as noted in the preceding explanations. It is requested that the Examiner reconsider his position and withdraw this rejection.

Rejections under 35 U.S.C. 102

Claims 25-37 and 123-128 are rejected as allegedly being anticipated by Lama *et al.* ("Lama"). The Examiner alleges that Lama discloses an identical preparation of the claimed enzyme.

Applicants respectfully traverse this rejection. Lama discloses an enzyme having thermostable amylolytic activity, and describes a preparation method for such an enzyme under the section "Preparation of the Enzyme." Following this method, Lama obtained his

intended enzyme by subjecting a *Sulfolobus solfataricus* strain to the series of steps described, i.e., cell cultivation, harvesting, cell disruption, ammonium sulfate precipitation and acetone precipitation steps. Lama et al. designated the enzyme thus obtained as Partially Purified Enzyme (PPE). The enzyme activity of such PPE can be reasonably assumed that, if the PPE were subjected to the activity measurement described in Example II-1 in the present specification, the enzyme shows a trehaloseoligosaccharide-hydrolyzing activity value, i.e., a specific activity value, of 3.45 units/mg (the value for the 60% saturated $(NH_4)_2SO_4$ precipitation shown in Table 12), 2.40 units/mg (the value for the crude extract shown in Table 13), or 5.97 units/mg (the value for the crude extract shown in Table 14), at the highest. On the other hand, the claimed amylase, i.e., the amylase claimed in amended claim 25 and newly added claim 150, has a specific activity of more than 10.6 units/mg. In this regard, the Examiner's attention is directed to the fact that the amylases obtained via the purification by DEAE ion-exchange chromatography in Example II-2, Example II-3 and Example II-4 in the present application have a specific activity of 253 units/mg, 10.6 units/mg and 101 units/mg, respectively.

In regard to the claims that are defined as comprising a specific amino acid sequence, the present purified amylase can be identified as a polypeptide comprising an amino acid sequence of SEQ ID NO: 6 or NO: 8, or can be identified as a polypeptide encoded by a nucleotide comprising a base sequence of SEQ ID NO: 5 or NO: 7. The Examiner has not provided any evidence that the PPE of Lama anticipates the claimed amylases.

In view of these arguments and further clarifications of the claimed amylase properties, it is requested that this rejection be withdrawn.

CONCLUSION

The present response is intended to be a complete response to the Examiner's Office Action. It is believed that the above arguments and amendments to the claims place the application in condition for allowance, and a notice to that effect is respectfully requested. If there are any minor issues which can be taken care by telephone, it is requested that the Examiner contact the undersigned attorney at telephone number below.

Respectfully submitted,

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MARKED-UP VERSION OF THE SPECIFICATION

Marked up version of the paragraph starting at page 23, lines 20-24 is below:

Fig. 31 is an illustration showing the homology between the amino acid sequence of the novel transferase derived from the *Sulfolobus solfatarius* strain KM1 **(SEQ ID NO: 2)** and that derived from the *Sulfolobus acidocaldrius* strain ATCC 33909 **(SEQ ID NO: 4)**.

Marked up version of the paragraph starting at page 23, lines 25-29 is below:

Fig. 32 is an illustration showing the homology between the base sequence of the gene coding for the novel transferase derived from the *Sulfolobus solfatarius* strain KM1 **(residues 455-2518 of SEQ ID NO: 1)** and that derived from the *Sulfolobus acidocaldrius* strain ATCC 33909 **(residues 816-2844 of SEQ ID NO: 3)**.

Marked up version of the paragraph starting at page 24, lines 21-24 is below:

Fig. 40 is an illustration showing the homology between the amino acid sequence of the novel transferase derived from the *Sulfolobus acidocaldrius* strain ATCC 33909 **(SEQ ID NO: 8)** and that derived from the *Sulfolobus solfatarius* strain KM1 **(SEQ ID NO: 6)**. **The full-length *Sulfolobus solfatarius* strain KM1 protein (prior to post-translational modification) coded for by SEQ ID NO: 5 is shown in SEQ ID NO: 63.**

Marked up version of the paragraph starting at page 24, lines 25-29 is below:

Fig. 41 is an illustration showing the homology between the base sequence of the gene coding for the novel transferase derived from the *Sulfolobus acidocaldrius* strain ATCC 33909 **(residues 1176-2843 of SEQ ID NO: 7)** and that derived from the *Sulfolobus solfatarius* strain KM1 **(residues 642-2315 of SEQ ID NO: 5)**.

Marked up version of the paragraph starting at page 149, line 34 to page 150, line 13 is below:

According to information about the partial amino acid sequences of the novel *Sulfolobus solfataricus* strain KM1, which is determined in Example I-9, oligonucleotide

DNA primers are prepared by using a DNA synthesizer (Model 381 manufactured by Applied Biosystems Co.). Their sequence were as follows.

DN-1

Amino Acid Sequence

N terminus AspGluPheArgGluSer C terminus **(SEQ ID NO: 59)**

DNA Primer 5' TTTACGAAAAACCTCATC 3' (S**EQ**~~equence~~ **ID** N~~o.~~**O:** 28)

Base Sequence C T TG T T

DN-8

Amino Acid Sequence

N terminus AspAsnIleGluTyrArgGly C terminus **(SEQ ID NO: 60)**

DNA Primer 5' GATAACATAGAATACAGAGG 3' (S**EQ**~~equence~~ **ID** N~~o.~~**O:** 29)

Base Sequence T T G T G

Marked up version of the paragraph starting at page 172, lines 12-29 is below:

According to information about the partial amino acid sequences determined in Example II-20, oligonucleotide DNA primers are prepared by using a DNA synthesizer (Model 381 manufactured by Applied Biosystems Co.). Their sequence were as follows.

AP-10

Amino Acid Sequence **(SEQ ID NO: 61)**

N terminus Pro Ala Ser Arg Tyr Gln Pro C terminus

DNA Primer 5' AGCTAGTGAGATATCAACC 3' (S**EQ**~~equence~~ **ID** N~~o.~~**O:** 57)

Base Sequence A G C C G

AP-11

..(complementary strand)

Amino Acid Sequence **(SEQ ID NO: 62)**

N terminus Asp Val Phe Val Tyr Asp Gly Lys C terminus

DNA Primer 5' TTTTCCATCATAAACAAAAACATC 3'

(S**EQ**~~equence~~ **ID** N~~o.~~**O:** 58)

Base Sequence C A G T G T

C

MARKED UP VERSION OF THE CLAIMS

25. (Amended) An [A novel] amylase which acts on a substrate saccharide, the substrate saccharide being composed of at least three sugar units wherein at least three sugar units from the reducing end are glucose residues, so as to liberate principally monosaccharides and/or disaccharides by hydrolyzing the substrate saccharide from the reducing end side, and shows a trehaloseoligosaccharide-hydrolyzing activity of more than 10.6 units/mg wherein 1 unit equals the activity of liberating 1 μmol of α,α-trehalose per hour from maltotriosyltrehalose.

26. (Amended) The [novel] amylase claimed in Claim 25 which has a principal activity of acting on a substrate saccharide, the substrate saccharide being composed of at least three sugar units wherein at least three sugar units from the reducing end side are glucose residues and the linkage between the first and the second glucose residues from the reducing end side is α-1, α-1 while the linkage between the second and the third glucose residues from the reducing end side is α-1,4, so as to liberate α,α-trehalose by hydrolyzing the α-1,4 linkage between the second and the third glucose residues.

27. (Amended) The [novel] amylase claimed in Claim 25, wherein said amylase also has an activity of endotype-hydrolyzing one or more α-1,4 linkages within the molecular chain of a substrate.

28. (Amended) The [novel] amylase claimed in Claim 25, wherein said amylase has an activity of hydrolyzing a substrate trehaloseoligosaccharide [such as glucosyl-trehalose and maltooligosyltrehalose] at the α-1,4 linkage between the second and the third glucose residues from the reducing end side to liberate α,α-trehalose.

29. (Amended) The [novel] amylase claimed in Claim 25, wherein its molecular weight measured by SDS-polyacrylamide gel electrophoresis is 61,000 to 64,000, approximately.

30. (Amended) The [novel] amylase claimed in Claim 25, wherein the amylase has the following physical and chemical properties:

(1) Optimum pH with in the range from 4.5 to 5.5;

(2) Optimum temperature within the range from 60 to 85°C;

(3) pH Stability within the range from [4.0] 3.0 to [10.0] 13.0; and

(4) Thermostability which allow 100% enzymatic activity to remain even after exposure at 80 to 85°C for 6 hours.

31. (Amended) The [novel] amylase claimed in Claim 25, wherein the isoelectric point measured by isoelectric focusing is pH 4.3 to pH 5.4.

32. (Amended) The [novel] amylase claimed in Claim 25, wherein its activity can be fully inhibited with 5 mM $CuSO_4$.

33. (Amended) The [novel] amylase claimed in Claim 25, wherein the amylase is derived from an archaebacterium belonging to the order *Sulfolobales*.

34. (Amended) The [novel] amylase claimed in Claim 33, wherein the amylase is derived from an archaebacterium belonging to the genus *Sulfolobus*.

35. (Amended) The [novel] amylase claimed in Claim 34, wherein the archaebacterium belonging to the genus *Sulfolobus* is the *Sulfolobus solfataricus* strain KM1 (FERM BP-4626) [or a variant thereof].

36. (Amended) The [novel] amylase claimed in Claim 34, wherein the archaebacterium belonging to the genus *Sulfolobus* is the *Sulfolobus solfataricus* strain DSM 5833 [or a variant thereof].

37. (Amended) The [novel] amylase claimed in Claim 34, wherein the archaebacterium belonging to the genus *Sulfolobus* is the *Sulfolobus acidocaldarius* strain ATCC 33909 [or a variant thereof].

123. (Amended) A polypeptide comprising an amino acid sequence [shown in Sequence No. 6 or an equivalent sequence thereof] of SEQ ID NO: 6.

124. (Amended) A polypeptide comprising an amino acid sequence [shown in Sequence No. 8 or an equivalent sequence thereof] of SEQ ID NO: 8.

126. (Amended) The polypeptide claimed in [Claim 123] any one of Claims 124, 152 or 153, which has an activity of acting on a substrate saccharide, the substrate saccharide being composed of at least three sugar units wherein at least three sugar units from the reducing end side are glucose residues and the linkage between the first and second glucose residues, from the reducing end side is α-1, α-1 while the linkage between the second and third glucose residues from the reducing end side is α-1,4, so as to liberate α,α-trehalose by hydrolyzing the α-1,4 linkage between the second and third glucose residues.

127. (Amended) The polypeptide claimed in [Claim 123] any one of Claims 124, 152 or 153, which has the following principal activities:

(1) An activity of endotype-hydrolyzing one or more of α-1,4 glucoside linkages in a sugar chain;

(2) an activity of acting on a substrate saccharide, the substrate saccharide being composed of at least three sugar units wherein at least three sugar units from the reducing end are α-1,4-linked glucose residues, so as to liberate principally monosaccharide and/or disaccharide by hydrolyzing the substrate from the reducing end side; and

(3) an activity of acting on a substrate saccharide, the substrate saccharide being composed of at least three sugar units wherein at least three sugar units from the reducing end side are glucose residues and the linkage between the first and second glucose residues from the reducing end side is α-1, α-1 while the linkage between the second and third glucose residues from the reducing end side is α-1,4, so as to liberate α,α-trehalose by hydrolyzing the α-1,4 linkage between the second and third glucose residues.

128. (Amended) The [polypeptide] amylase claimed in Claim [123] 25, wherein the optimum temperature for its action is 60 to 85°C.